Effects of Colestipol-Niacin Therapy on Human Femoral Atherosclerosis

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The 2-year therapy effect on femoral atherosclerosis was evaluated in the Cholesterol Lowering Atherosclerosis Study (CLAS), a randomized, placebo-plus-diet-controlled angiographic trial of colestatipol-niacin therapy plus diet in men with previous coronary bypass surgery. Different diet compositions were prescribed to enhance the differential in blood cholesterol responses between the two groups. The annual rate of change in computer-estimated atherosclerosis (CEA), a measure of lumen abnormality, was evaluated between treatment groups. A significant per-segment therapy effect was found in segments with moderately severe atherosclerosis ($p < 0.04$) and in proximal segments ($p < 0.02$). When segmental CEA measures were combined into a per-patient score using an adaptation of the National Heart, Lung, and Blood Institute scoring procedure, a significant therapy effect was observed ($p < 0.02$). Total variance of the annual change rate in CEA was as predicted from pilot studies, but measurement variation was larger. The therapy effect observed in femoral arteries, although significant, was less marked than the strong and consistent benefit previously reported for both native coronary arteries and aortocoronary bypass grafts. (Circulation 1991;83:438–447)

Blood cholesterol lowering has been shown to significantly reduce clinical events secondary to coronary atherosclerosis and to have beneficial effects on coronary angiographic status. Effects of blood cholesterol lowering in other vascular beds such as the cervicocerebral, iliofemoral, and aorta have received less attention; among these vessels, effects are least known about the femoral artery. There are no autopsy-derived estimates of spontaneous femoral atherosclerosis change in contrast to those that are available for coronary, cervicocerebral, and aortic lesions. Current knowledge of the natural history of femoral atherosclerosis is largely derived from two retrospective studies of patients with advanced symptomatic disease. The reported angiographic observations on blood cholesterol lowering and femoral atherosclerosis are confined to two uncontrolled case series, two small controlled clinical trials, and preliminary reports from a trial in progress. These reports indicate favorable therapy effects in hyperlipoproteinemia and in symptomatic patients with advanced femoral disease.

The Cholesterol Lowering Atherosclerosis Study (CLAS) was a randomized, placebo-plus-diet-controlled trial of aggressive therapy to reduce blood low density lipoprotein (LDL) cholesterol level and increase blood high density lipoprotein (HDL) cholesterol level with colestatipol-niacin therapy plus diet. Target vessels were chosen to provide as complete a survey of atherosclerosis as possible, consistent with patient safety. Femoral, coronary, and cervical vessel beds were visualized in 162 patients at baseline and after 2 years of treatment (CLAS-I) and in a subset of 101 patients after 4 years (CLAS-II).

We have previously reported a strong 2-year therapy effect in coronary arteries and coronary bypass grafts in CLAS patients. The 2-year therapy effects in the femoral arteries as determined from computed angiographic analysis are reported. The computed estimate of atherosclerosis (CEA—see below) was the primary end point of CLAS and was used in 1975 in determining the CLAS sample size. The coronary end point using a panel assessment was instituted for safety reasons and was the end point reported in Reference 3.
Methods

Study Groups and Experimental Design

The CLAS study design has been described. Briefly, CLAS was a randomized, angiographic trial testing the therapy effect of colesterol hydrochloride and niacin plus diet (experimental group) versus placebo plus diet (control group) in 162 of 188 randomized nonsmoking men aged 40–59 years who had progressive atherosclerosis and previous coronary bypass surgery (80 men in the experimental group versus 82 in the control group). Both groups received diet intervention with different diet composition to enhance the differential in blood cholesterol responses between the two groups (30–40% decrease in the experimental group, and 7–8% decrease in the control group). The target diet prescribed for the control group included a daily intake of less than 250 mg cholesterol and provided 26% of energy as fat calories, 10% as polyunsaturated fat, and 5% as saturated fat. The target diet prescribed for the experimental group included a daily intake of less than 125 mg cholesterol and provided 22% of energy as fat calories, 10% as polyunsaturated fat, and 4% as saturated fat.

Entry fasting blood cholesterol levels were in the range of 185–335 mg/dl. Average (±SEM) age at entry of all patients was 54.2±0.5 years, and average blood pressure was 124±2/81±1 mm Hg. Forty-one percent of experimental patients and 37% of control patients were treated for mild hypertension, principally with β-adrenergic blockers. Twenty-nine percent of all patients never smoked, and 71% were exsmokers for more than 6 months. Levels of physical activity were judged as moderate from questionnaires, records of caloric intake, and serial measurements of body weight.

Patients were evaluated angiographically before randomization to obtain baseline data on the atherosclerotic disease of the carotid, femoral, and coronary arteries as well as of the coronary bypass grafts. Patients were then followed at specified intervals for 2 years, at which time a repeat angiogram was performed.

The primary reasons for dropping out of the study were refusal to have a second angiogram (four in each group), dislike of medication (five in experimental group and two in control group), health-related reasons (one experimental patient with congestive heart failure and one control patient with sudden cardiac death), administrative disagreement over hospital charges (one in each group), move out of state (one in each group), missed study visits (two in experimental group), work related (one in control group), lost angiogram (one in control group), and attitude after discovery of smoking recidivism (one in control group).

Lipid Analysis

All blood samples were obtained after patients fasted overnight (8 hours or longer). Total cholesterol, triglycerides, and HDL cholesterol isolated by precipitation of the low-density species were analyzed by Auto Analyzer II methodology and standardized against reference materials supplied by the Standardization Program of the National Centers for Disease Control. For blood samples with triglyceride values of less than 500 mg/dl, LDL cholesterol was calculated as follows:

\[
LDL\text{ cholesterol} = (\text{total cholesterol} - \text{HDL cholesterol}) - \frac{\text{triglycerides}}{5}
\]

Total cholesterol, triglycerides, and HDL cholesterol were measured at each screening visit, at monthly intervals for the first 6 months on treatment, and at 2-month intervals thereafter.

Baseline averages for each blood lipid and lipoprotein were the unweighted values obtained at the first three screening visits. On-trial averages were obtained from values weighted according to the scheduled interval (either 1 or 2 months) between treatment visits.

Angiographic Analysis

Bilateral femoral angiography was performed using radiographic factors previously shown by tests in human cadavers to produce accurate measures of femoral artery pathology. The in vivo reproducibility of femoral atherosclerosis measures was tested in a previous pilot study. In CLAS, coronary, femoral, and cervical artery visualizations were performed in the same sequence at all examinations. A description of the entire procedure has been published, and details relevant to femoral visualization are given here.

Patients were fasting and were not premedicated. The right femoral artery (rarely, the left femoral artery) was punctured with a one-piece Cook needle and a J wire was advanced to the descending aorta. Next, a pigtail catheter was introduced into the descending aorta, where it was cleared and connected to pressure recordings. After 1982, an arterial introducer (USCI) was used. Then, 5,000 IU heparin was given through the intra-arterial catheter, which was advanced to the ascending aorta, and cervical angiography was performed.

The pigtail catheter was withdrawn to the abdominal aorta, just above the bifurcation. Next, an image intensifier was positioned above the knee, and the timing of arrival of Renografin 76 to Hunter's canal was established by injecting 4 ml of contrast medium at 30 ml/sec. Femoral angiography was then performed by injecting 40 ml of contrast medium at 30 ml/sec, and films were exposed at a rate of 3 sec⁻¹ for 5 seconds. A Schonander serialogram with du Pont DBS Quanta 3 screens was used. The radiographic source focal spot size was 0.6×1.3 mm. Constant tube to film distance of 100 cm was maintained throughout the study. Typical exposure factors were KV 60-66 and MAS 20-32. A step wedge filled with contrast medium was placed between the patient's legs and radiographed. Control of film processing was through measurement of film densities in the phantom in
comparison with completely exposed and nonexposed film densities.

Films for analysis were selected by an angiographer with treatment status masked. Branch points on the femoral angiogram were marked and used for registration, duplicating the procedure used in pilot studies of replication.19

Film digitization was carried out with a Joyce Loebl SCANDIG 3 rotating drum digitizer. In this instrument, a tungsten halogen lamp is mounted opposite a photodiode on an optical carriage that moves along the axis of the transparent rotating drum. All picture elements are scanned with the same light source and sensor combination, and variation in illumination of each pixel is virtually zero. Density linearity of this system was ±3 parts in 256 from a best-fit straight line. Density repeatability was ±1 part in 256.

Image Processing

Image processing involved 1) finding the vessel edge, 2) calculating the midline, and 3) correcting for curvature. In brief, the following steps were performed (see Reference 14 for details).

Vessel edge finding. Left and right vessel edges were determined for each horizontal row of image data. Fifty-micron digital sampling was used to convert a 2.5 × 5.0-cm segment of the angiogram to a 500 × 1,000-element digital array. Because the vessel orientation was approximately vertical in the digitized image, this procedure was designed to find edges along lines orthogonal to the vessel midline. Where vessel curvature existed, the rows of image data were not orthogonal to the midline, and estimators of vessel width were too large. During the initial steps of edge tracking, this error was ignored and vessel curvature was measured. If the curvature exceeded a threshold (described below), a geometric transformation was used to straighten the vessel and edge tracking was repeated.

To find the edges, the gradients of picture intensity were calculated for a sequence of points in each row of the image. The gradient value was obtained with a linear filter that combined image smoothing and differentiation. This filter was implemented as a weighted average of 43 picture points. The window for the first line was set manually. The gradients were weighted to give greater importance to gradients that occurred near the center of the window. The left and right vessel edges were defined as the coordinates of the weighted maximum positive and negative gradients, respectively. Left and right edges were found independently.

At some places along the vessel, such as at the origin of larger branches, the edges were ambiguous and the edge tracking was unreliable. At these places, the computer operator indicated the position of the bad edges with a cursor, and these points were subsequently ignored when measuring vessel characteristics.

Midline calculation. After all valid edges have been found, missing edges due to branches or crossing vessels were temporarily filled in by linear interpolation from the nearest valid edges. The midpoint between the left and right edges for each line was determined, and the set of midpoints was then smoothed using a 401-point averaging filter.

Curvature correction. Curvature of the vessel was estimated by fitting a minimum least-squares straight line to the midline and defining a curvature index (CI) as the root-mean-square difference between this line and the midline points. Segments with CI of more than 10 pixels were straightened by resampling the original digitized image to the vessel midline over the 5-cm film segment plus 100 lines at the top and bottom to allow change in segment length during straightening. This procedure was tested in a random sample of films and found to eliminate outlying values of CEA in curved vessels. Picture elements of each straightened line were obtained using bilinear interpolation. The midline in the straightened image was exactly vertical.

Computer Measures of Atherosclerosis

Four measures of atherosclerotic lesion characteristics were derived.19,20 The first, the vessel width, was defined as the average difference between the right and left edge coordinates. The second, R(81,97), and third, R(97,321), were roughness measures obtained by comparing the effects of a long and short filter on edge points.18 For example, R(97,321) was obtained from a comparison of a 97-point edge-smoothing filter with a 321-point edge filter.

The fourth measure, computer-estimated atherosclerosis (CEA), was calculated as a (dimensionless) linear function of the tapered lumen 90 (TL90) as follows.14,20 First, a vessel width was computed for each image line, and a least-squares straight line was fit to the sequence of width values as a means of estimating vessel taper. If s is the slope of the line fit to the widths, then \( w = s \times \sqrt{\text{image height}} \) is an estimate of the change in vessel width due to taper. The slope computation excluded lines for which the left or right edges were missing. Next, the midline was tilted by one half of \( w \) due to taper and then translated to the right until 90% of the right-hand edge points lay to the left of the translated midline and 10% to the right. A similar process was applied to the left side of the vessel. The pair of translated midlines comprised the computer estimate of the predisuse lumen location. TL90 is defined to be the root-mean-square difference between the detected edge and the tilted translated midlines. This quantity was computed for 5-cm segments as well as for 1-cm subsegments and for each side of a segment. Areas of missing edges were excluded from the computation. Finally, CEA is defined as

\[
\text{CEA} = -3.34 + 0.179 \times \text{TL90}
\]

The linear relation between CEA and TL90 was derived through a postmortem study that calibrated the method with 128 5-cm human femoral artery segments.18
The computer estimate of the diseased lumen incorporated in CEA is independent of vessel dilation because the location of the left and right computer lumen lines are determined independently of each other. The shape of the computer lumen is derived from the midline of the detected edges, which is independent of vessel dilation. After the lumen shape is determined, it is translated to the left until 10% of the edge points are to the left of the lumen and 90% are to the right. A symmetrical process is used to find the location of the computer lumen on the right. Thus, the locations of the left and right lumens are determined independently of each other and of vessel width.

These measures were tested against a ranked series of atherosclerotic femoral arteries obtained at autopsy. Segmental width (mm) was found to be a poor correlate of atherosclerosis (correlation equals 0.034 for vessel wall cholesterol content, \( p=NS \); correlation equals \(-0.196 \) for visible atherosclerosis in autopsy specimen, \( p=NS \)). On the other hand, both roughness measures were found to be satisfactory measures of atherosclerosis. For example, \( R(81,97) \) was shown to be an independent predictor of atherosclerotic lesion characteristics, and the correlation of \( R(97,321) \) with vessel wall cholesterol content was 0.702 \((p<0.001)\) and with the extent of visible atherosclerosis in autopsy specimens was 0.739 \((p<0.001)\). However, CEA was found to be the most robust measurement under conditions of clinical angiography. Consequently, it was adopted as the primary femoral measure in CLAS, and the annual change rate in CEA was the end point for determining the power of the CLAS trial.

To determine the annual change rate in CEA for CLAS patients, films were cut and coded in a manner that masked the order of examination and treatment assignment from the computer operator. During film cutting and coding, a reference landmark visible in both members of the film pair (usually the origin of a small branch) was indicated with a marking crayon. The scanned 5-cm segment areas were located in such a manner that the corresponding vessel segment on the other angiogram in the matched pair was included in the same segment when the second film was scanned.

**Sample Size and Power**

Sample size determinations, based on the annual change rate in CEA, required 160 patients (of 188 randomized into one of two treatment arms) to complete 2 years of follow-up. This was to provide a power of 0.80 to detect a “moderate” therapy effect (40%) in the annual change rate in CEA at the 0.05 level against a one-sided alternative. These projections were based on previous experience with atherosclerosis measurements in change in CEA obtained in 5-cm femoral segments and averaged to obtain a per-patient end point. Assuming a components-of-variance model with factors accounting for subject to subject variability as well as measurement error, we calculated maximum likelihood estimates of these data. The expected variation in atherosclerosis change from subject to subject (14.1) and anticipated error of measurement of change per subject (14.4) were of equal magnitude. The overall variance used in the power calculations was \( V = 14.1 + 14.4 / T^2 = 17.7 \), where \( T \) of 2 years is the interangiographic interval. Detecting a therapy effect of 40% required a differential of 1.68 CEA units/yr between the two treatment groups.

**Extent of Masking**

Both study patients and clinic staff were masked to the prerandomization study drug trial lipids. Study patients were masked to treatment assignment. During follow-up, clinic personnel were not masked to treatment assignment, and patients and clinic staff were not masked to on-trial lipid values. Evaluation of study outcome measures (panel assessment of coronary status and CEA) were carried out by staff and consultants who were masked to treatment group assignment as well as temporal ordering of angiographic data; this included the angiographer as well as the coronary panel and staff determining femoral CEA.

**Statistical Analysis**

Analyses of the annual change rate in CEA were performed on both a per-segment and a per-patient basis. For the per-segment analysis, we used the method of Rossner. This method permits testing for treatment efficacy on a per-segment basis after adjusting for the within-subject correlation. Analyses were performed overall and within quartiles of baseline CEA and for proximal and distal segments. The per-segment evaluation was part of the original study design.

For the per-patient analysis of CEA, we used an adaptation of the National Heart, Lung, and Blood Institute (NHLBI) scoring procedure. The per-patient analysis was not part of the original analytic plan. The NHLBI scoring procedure, established in 1984, presented an appropriate method for scoring segments to arrive at a per-patient outcome. On the basis of the distribution of the annual change in CEA for both groups, we classified segments as progressing, regressing, or not changing. We classified a segment as regressing if it was less than 1 SD below the mean annual change rate in CEA. A segment progressed if it was greater than 1 SD above the mean annual change rate in CEA. Otherwise, there was no change in the segment. The mean and SD were to be determined empirically from the distribution of the annual change rate in CEA for CLAS patients. We then evaluated overall patient femoral status using the following algorithm. A progressor was defined as a patient with progression in at least one segment and no change in the others. A regressor was defined as a patient with regression in at least one segment and no change in the others. A patient was regarded as having demonstrated no change if all segments exhibited no change. Patients who had some segments that regressed and others that pro-
gressed were classified on the basis of the modal segmental response. Thus, if the majority of the segments progressed but some regressed (and possibly some did not change), a patient was classified as a regressor. On the other hand, if the majority regressed and some progressed, that patient was classified as a regressor. Finally, if equal numbers of segments regressed and progressed, the patient was classified as having not changed. Analysis of the association of treatment with patient status (progression/regression) was performed using the Fisher's exact test. These analyses were also stratified by baseline cholesterol levels.

We evaluated the patient scoring procedure with the other image processing measures: vessel width and edge roughness \[R(81,97), R(97,321)\]. We used analysis of variance procedures to test for significance in the average annual change rates in vessel width, \(R(81,97)\), and \(R(97,321)\) across the three patient groups defined by regressor-regressor femoral status. Because there were different numbers of segments per patient, we weighted these analyses by the numbers of segments. In addition, on-trial lipid levels were compared across the three patient groups. For all analyses, when significant differences were obtained, pairwise comparisons were made using a Bonferroni adjustment.

**Results**

Seventy-seven patients (of 80) in the experimental group and 75 patients (of 82) plus one patient (of 26) who did not have coronary end point data (and was not included in our previous coronary report) in the control group had segments that were analyzable at baseline and at 2 years.

Table 1 gives a summary of baseline and on-trial determinations of fasting blood lipids. There were no significant differences between the treatment groups at baseline. When comparing the on-trial values between the two treatment groups, large significant differences were found for all lipids \((p<0.001)\). Within the experimental group, statistically significant percent decreases from baseline were found for total cholesterol \((26\% -\text{percent not shown in Table} 1)\), triglycerides \((22\%)\), LDL cholesterol \((43\%)\), ratio of LDL cholesterol to HDL cholesterol \((57\%)\), and ratio of total cholesterol to HDL cholesterol \((45\%)\). In addition, the drug and diet therapy significantly increased HDL cholesterol \((38\%)\). In the control group, modest yet statistically significant decreases were found for total cholesterol \((4\%)\), triglycerides \((5\%)\), LDL cholesterol \((5\%)\), ratio of LDL cholesterol to HDL cholesterol \((6\%)\), and ratio of total cholesterol to HDL cholesterol \((5\%)\). HDL cholesterol did not change significantly.

**Per-Segment Analysis**

The average number of pre/post pairs that were analyzed was 6.8 per patient for the experimental group and 6.9 per patient for the control group. The two treatment groups were on trial for an average of 2.05 (experimental group) and 2.06 years (control group). The two treatment groups showed similar femoral disease at baseline as measured by baseline CEA (Table 2). Overall, each group exhibited significant progression \((p<0.05)\); although the control group demonstrated slightly larger progression than the experimental group, this was not statistically significant.

Table 2 also summarizes CEA change rates for segments categorized into quartiles on the basis of the cumulative distribution of baseline CEA. On the average, for segments with little femoral disease (quartiles 1 and 2), there was significant increase in CEA per year within each treatment group but no differences between treatment groups. For segments with moderate and large femoral disease (quartiles 3 and 4), there was no significant change in CEA per year within each treatment group, although there was a trend toward regression in the experimental group (quartiles 3 and 4) and the control group (quartile 4). For quartile 3 (moderate disease), there was a significant difference between the treatment groups \((p<0.04)\); drug and diet therapy reduced the rate of progression, but placebo and diet therapy did not. The estimates of intersegment correlation ranged from 0.11 to 0.28.

Finally, Table 2 gives a summary of CEA change by distal and proximal segments. Baseline CEA was significantly greater for the distal segments than for the proximal segments \((p<0.0001)\). After treatment, there were no significant changes in CEA for the distal segments. For the proximal segments, there was a significant therapy effect \((p<0.02)\), with a significant increase in CEA per year noted in the control group \((p<0.001)\).

**Per-Patient Analysis**

We plotted the distribution of the annual change rate in CEA for all segments in both treatment

**Table 1. Mean (SEM) Baseline and On-Trial Fasting Lipid Levels by Treatment Group**

<table>
<thead>
<tr>
<th>Lipid (mg/dl)</th>
<th>Group</th>
<th>Baseline</th>
<th>On-trial</th>
<th>(p^t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>X</td>
<td>245 (4)</td>
<td>179 (3)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>242 (4)</td>
<td>232 (4)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>X</td>
<td>153 (9)</td>
<td>115 (5)*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>154 (10)</td>
<td>142 (9)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>X</td>
<td>44 (1)</td>
<td>60 (1)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>44 (1)</td>
<td>44 (1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>X</td>
<td>170 (3)</td>
<td>96 (3)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>168 (4)</td>
<td>160 (3)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>X</td>
<td>4.0 (0.1)</td>
<td>1.7 (0.1)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>X</td>
<td>4.0 (0.1)</td>
<td>3.8 (0.1)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>X</td>
<td>5.7 (0.2)</td>
<td>3.1 (0.1)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>X</td>
<td>5.7 (0.1)</td>
<td>5.4 (0.1)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Non-HDL cholesterol</td>
<td>X</td>
<td>201 (4)</td>
<td>119 (3)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>199 (4)</td>
<td>188 (4)*</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein; LDL, low density lipoprotein; X, niacin-colestipol plus diet (30% cholesterol reduction) \((n=77)\); C, placebo plus diet (7% cholesterol reduction) \((n=76)\).

*Significant change from baseline \((p<0.001)\).

†Comparing on-trial lipids between treatment groups.
TABLE 2.  Femoral Computer-Estimated Atherosclerosis (CEA) at Baseline and Annual Change Rate in CEA by Treatment Group, Baseline CEA Quartiles, and Distal/Proximal Segments

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number of segments</th>
<th>Number of patients</th>
<th>Mean CEA (SEM)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Baseline</td>
<td>Annual change rate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Within groups</td>
<td>Between groups</td>
</tr>
<tr>
<td>All segments</td>
<td>X</td>
<td>533</td>
<td>40.3 (0.9)</td>
<td>0.05 NS</td>
</tr>
<tr>
<td>C</td>
<td>518</td>
<td>77</td>
<td>40.4 (0.9)</td>
<td>0.0001 &lt;0.001</td>
</tr>
<tr>
<td>1st quartile†</td>
<td>X</td>
<td>135</td>
<td>22.7 (0.3)</td>
<td>NS &lt;0.0001 NS</td>
</tr>
<tr>
<td>C</td>
<td>149</td>
<td>53</td>
<td>22.5 (0.3)</td>
<td>NS &lt;0.0001 NS</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>X</td>
<td>144</td>
<td>31.4 (0.2)</td>
<td>NS &lt;0.0001 NS</td>
</tr>
<tr>
<td>C</td>
<td>115</td>
<td>60</td>
<td>31.3 (0.2)</td>
<td>NS &lt;0.0001 NS</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>X</td>
<td>128</td>
<td>41.7 (0.3)</td>
<td>NS &lt;0.04 NS</td>
</tr>
<tr>
<td>C</td>
<td>124</td>
<td>59</td>
<td>41.4 (0.3)</td>
<td>NS NS</td>
</tr>
<tr>
<td>4th quartile</td>
<td>X</td>
<td>126</td>
<td>67.9 (1.9)</td>
<td>NS NS</td>
</tr>
<tr>
<td>C</td>
<td>130</td>
<td>52</td>
<td>67.9 (1.6)</td>
<td>NS NS</td>
</tr>
<tr>
<td>Distal</td>
<td>X</td>
<td>155</td>
<td>44.9 (1.6)</td>
<td>NS NS</td>
</tr>
<tr>
<td>C</td>
<td>142</td>
<td>76</td>
<td>43.9 (1.7)</td>
<td>NS NS</td>
</tr>
<tr>
<td>Proximal</td>
<td>X</td>
<td>154</td>
<td>33.4 (1.0)</td>
<td>NS &lt;0.02 NS</td>
</tr>
<tr>
<td>C</td>
<td>141</td>
<td>76</td>
<td>33.9 (1.3)</td>
<td>&lt;0.001 NS</td>
</tr>
</tbody>
</table>

X, niacin-colestipol plus diet (30% cholesterol reduction); C, placebo plus diet (7% cholesterol reduction).

*Analysis using the modeling approach due to Rosner22 against a one-sided alternative.

†CEA range: quartile 1 (11-27), quartile 2 (28-35), quartile 3 (36-48), quartile 4 (49-180).

The mean CEA change rate was 1.0, and the SD was 6.8. We classified a segment as having regressed if the change rate in CEA was less than 1.0 - 6.8 = -5.8. We classified a segment as having progressed if the change rate in CEA was more than 1.0 + 6.8 = 7.8. A segment with a change rate in the range (-5.8, 7.8) was regarded as having not changed. We then calculated the overall patient femoral status using the algorithm described above.

Figure 2 (left panel) shows the distribution of regressors, nonchangers, and progressors for each treatment group. Fifty-six of 153 patients (37%) regressed, a larger proportion being in the experimental group (35 of 77, or 45%) than in the control group (21 of 76, or 28%) (p = 0.02 using a one-sided Fisher’s exact test comparing the rate of regression with that of progression or not changing). On the other hand, 51 of 153 patients (33%) progressed, a smaller proportion being in the experimental group (21 of 77, or 27%) than in the control group (30 of 76, or 39%) (p = 0.08 comparing the rate of progression with that of regression or not changing). Analysis of the 2x2 table comparing regressors with progressors (eliminating nonchangers) revealed that the rate of regression was significantly greater in the experimental group (35 of 56, or 63%) than in the control group (21 of 51, or 41%) (p = 0.02).

We then stratified by baseline cholesterol levels (Figure 2, right panels). For patients with cholesterol of 240 mg/dl or less, 15 of 22 experimental patients (68%) showed regression compared with 11 of 28 control patients (39%) (p < 0.04); for patients with cholesterol of more than 240 mg/dl, 20 of 34 experimental patients (59%) showed regression compared with 10 of 23 control patients (43%) (p = NS).

Using these classifications, we compared the other angiographic image processing measures with patient status. Annual changes in R(81,97) and R(97,321) were significantly correlated with progression/regression (p < 0.0001), but change in vessel width was not (Table 3).

We also compared on-trial lipid determinations with patient status (Table 3). (These analyses were performed after noting that the distribution of age at randomization and baseline lipids were not significantly different across the three groups.) HDL cholesterol and ratio of total cholesterol to HDL cholesterol were associated with patient status (p ≤ 0.05). In addition, ratio of LDL cholesterol to HDL cholesterol was marginally significant (p < 0.06). Overall,
FiguRe 2. Bar graphs of percent distribution of progressors, nonchangers, and regressors by treatment. Solid bars represent drug and diet group (experimental), and striped bars represent placebo and diet group (control). Numbers above bars indicate number of patients. See text for definitions of regressors, progressors, and nonchangers. Top right panel is similar to left panel for patients with baseline total cholesterol of 240 mg/dl or less. Bottom right panel is similar to left panel for patients with baseline total cholesterol of more than 240 mg/dl.

Regressors had larger HDL cholesterol values and smaller ratios of LDL cholesterol to HDL cholesterol and of total cholesterol to HDL cholesterol than patients who did not change femoral status. After a Bonferroni adjustment, statistical significance was obtained for only HDL cholesterol ($p<0.05$).

**Table 3. Patient Status in Relation to On-Trial Lipids and Annual Change Rates in Femoral Measures**

<table>
<thead>
<tr>
<th>Patient femoral status</th>
<th>Regressor ($n=56$)</th>
<th>No change ($n=46$)</th>
<th>Progressor ($n=51$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SEM) Annual Change Rate in Femoral Measure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEA</td>
<td>$-1.8 (0.6)^a$</td>
<td>$0.8 (0.6)^b$</td>
<td>$4.1 (1.0)^{ab}$</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>66 (69)</td>
<td>104 (84)</td>
<td>82 (78)</td>
<td>NS</td>
</tr>
<tr>
<td>R(81,97)</td>
<td>$-0.2 (0.2)^a$</td>
<td>$0.1 (0.2)$</td>
<td>$0.7 (0.3)^a$</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>R(97,321)</td>
<td>$-1.6 (1.3)^a$</td>
<td>$-0.2 (1.0)$</td>
<td>$4.2 (1.9)^a$</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Mean (SEM) On-Trial Lipid Level (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>199 (5)</td>
<td>206 (6)</td>
<td>212 (6)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>118 (6)</td>
<td>133 (11)</td>
<td>129 (11)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>56 (2)$^a$</td>
<td>49 (2)$^a$</td>
<td>52 (2)</td>
<td>0.05</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>120 (5)</td>
<td>130 (6)</td>
<td>135 (6)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol/HDL cholesterol</td>
<td>2.4 (0.2)</td>
<td>2.9 (0.2)</td>
<td>2.9 (0.2)</td>
<td>0.06</td>
</tr>
<tr>
<td>Total cholesterol/ HDL cholesterol</td>
<td>3.8 (0.2)</td>
<td>4.5 (0.2)</td>
<td>4.5 (0.2)</td>
<td>0.05</td>
</tr>
<tr>
<td>Non-HDL cholesterol</td>
<td>144 (6)</td>
<td>157 (7)</td>
<td>161 (7)</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^a$b Denotes groups that are significantly different on pairwise comparisons with a Bonferroni adjustment ($p<0.05$).

**Discussion**

**Therapy Effects in Femoral Atherosclerosis**

Previous studies. This is the first controlled trial to report a significant per-patient benefit in the femoral artery from blood lipid-lowering therapy. Previous
reports have been from uncontrolled case series and small trials not reporting a per-patient effect. In the first reported angiographic case series, Ost and Stenson\(^9\) treated 31 “hyperlipidemic” men for intermittent claudication with 3–6 g/day nicotinic acid. After a mean treatment period of 42 months, three patients showed regression and 11 patients demonstrated nonprogression in femoral lesions. Baseline and on-trial blood lipid levels were not reported. In a second case series from this laboratory, Barndt et al\(^10\) reported on 25 hyperlipidemic patients without symptomatic vascular disease. After an average of 13 months of treatment with lipid-lowering drugs, principally clofibrate and neomycin, nine patients showed regression and three demonstrated nonprogression. Regression of disease was significantly correlated with reductions in total cholesterol and triglyceride levels and systolic and diastolic blood pressures.

Olsson et al\(^12\) reported preliminary findings in eight asymptomatic patients with elevated blood lipid levels. Fenofibrate and nicotinic acid treatment produced a 39% reduction in total cholesterol, 64% reduction in total triglycerides, 82% decrease in very low density lipoprotein (VLDL)-triglycerides, 45% decrease in LDL-cholesterol, and a 30% increase in HDL-cholesterol. Analysis by computerized image processing\(^13\) demonstrated regression in femoral atherosclerotic lesions in six of eight patients as evidenced by reduction in plaque area.

A completed study of more advanced femoral artery disease reported by Duffield et al\(^11\) described quantitative evidence of disease stabilization and regression. Twenty-four patients with stable intermittent claudication and baseline total cholesterol of more than 252 mg/dl and/or triglycerides of more than 158 mg/dl were randomly assigned to a usual-care of drug treatment group that included dietary advice and cholestyramine, nicotinic acid, or clofibrate. All patients received antismoking advice and, if indicated, a weight-reducing diet. Patients in the usual-care group showed no significant lipid changes, whereas patients in the treatment group exhibited mean plasma reductions in total cholesterol (25%), triglycerides (45%), LDL cholesterol (28%), and VLDL cholesterol (57%) and a 26% increase in HDL cholesterol. Paired, matched femoral angiograms (obtained at an average of 19 months apart) were analyzed visually and by computed image processing. The treatment group had 60% fewer progressing arterial segments compared with the control group \(p<0.01\). The mean increment in the arterial surface area covered by plaque \(\text{mm}^2/\text{segment/yr}\) among the treated patients was 33% less than that observed among the nontreated patients \(p<0.01\). Regression, as determined by an edge irregularity index, was observed in 15 of 46 segments in the treatment group compared with seven of 46 in the control group \(p<0.05\). Although a per-patient therapy effect was not demonstrated, this encouraging result indicated that therapy affected the process of atherosclerosis on a per-segment basis.

CLAS. The drug and diet therapy effect observed in femoral arteries of CLAS patients was significant when analyzed per patient on the basis of consistency of change in CEA (end point measure used to plan for the number of study patients) and of computer-estimated edge roughness previously shown by calibration at autopsy to be a measure of atherosclerosis independent of CEA (Tables 2 and 3). To arrive at a per-patient end point, we applied the classification of the NHLBI Type II study\(^23\) to the computer-derived segmental femoral change rates for a per-patient treatment outcome measure that accommodated within-subject variation. CLAS femoral data classified on a three-step NHLBI scale showed a significant drug and diet therapy effect when comparing regressors with progressors (Figure 2). A drug and diet therapy effect was also noted for regression/progression rates for patients with lower baseline cholesterol levels \((\leq 240 \text{ mg/dl})\). Because CLAS patients were selected on the basis of previous aorto-coronary bypass surgery; had blood lipid levels typical of coronary disease, not hyperlipoproteinemia; had no symptoms of femoral artery disease; and were not tested with clinical measures of blood flow or flow reserve in the legs, it is not possible to relate the CLAS femoral artery outcome to previous femoral angiographic study results. Furthermore, because the diet compositions were different between the two treatment groups, it is not possible from these data to attribute the observed therapy effects to the drug alone, although it is likely that the drug was the primary contributor.

The femoral therapy effect in CLAS appears less marked than the strong and consistent benefit that was observed in both native coronary arteries and aorto-coronary bypass grafts during the preplanned visual coronary monitoring as dictated by safety and ethical concerns. The coronary effect led to truncation of the trial at 2 years. Although the femoral effect is also significant, it probably would not have led to trial truncation, both because of its lesser consistency and magnitude and because of the smaller threat to life and welfare posed by femoral atherosclerosis.

**Variability in CEA**

When planning CLAS in 1975, analysis of 160 patients provided a power of 0.80 to detect a 40% treatment effect in the annual change rate in CEA at the 0.05 level against a one-sided alternative.\(^14\) Using estimates of variation in atherosclerosis change from subject to subject \((14.1)\) and anticipated error of measurement of change per subject \((14.4)\), we obtained an overall variance estimate \((17.7)\). The effect size of 40% translated into a differential of 1.68 CEA units/yr between the two treatment groups.

The actual experience with femoral angiography in CLAS showed person-to-person variation to be smaller \((8.0)\), whereas the measurement variation was larger \((37.3)\). It is interesting, however, that the overall value for variance of \(8.0+37.3/4=17.3\) is essentially the same as that calculated from our previ-
ous study, and detection of an effect size of 40% required a differential of 1.66 CEA units/yr. A differential effect of 1.8=0.9=−(0.9) CEA units/yr was noted for segments with baseline CEA in the third quartile (Table 2) but not in other quartiles or in all patients combined. In addition, a differential effect of 1.9=2.6=−(0.7) was noted for proximal segments.

The larger measurement variation encountered in CLAS may be due to differences in radiographic technique between CLAS and our pilot study. For CLAS angiograms, both legs were filled simultaneously with contrast medium injected into the aorta. In our pilot study, contrast medium was injected through a needle into one femoral artery. Electrocardiographic gating was not performed in either study. Recent experiments in Sweden in which the performance of femoral angiography has been rigorously evaluated in duplicate procedures at 10-minute intervals indicate that the precision of measurement is significantly improved by exposures gated against the cardiac cycle.

**Femoral Artery Disease Risk Factors**

Criqui and coworkers, using noninvasive atherosclerosis-related flow measures, found epidemiological evidence that disease in large femoral vessels was significantly associated with cigarette pack-years, systolic blood pressure, and fasting blood glucose but not with blood lipoprotein levels, except for a marginal association of HDL cholesterol with severe disease. CLAS patients were nonsmokers and normotensive. In CLAS, therapy reduced blood lipid levels that may be principal coronary risk factors. Thus, the femoral outcome in CLAS is in accord with Criqui et al's findings.

It is possible that the significantly higher levels of HDL cholesterol in femoral regression patients compared with femoral progression patients may be an indication of differences between femoral and coronary arteries because HDL cholesterol was not found to have a significant effect in the coronary arteries. However, it is important to note that different measures of atherosclerosis change have been used in the two vessel beds (e.g., CEA change in femoral arteries and global coronary change score from human panelists in coronary vessels). We are now evaluating CLAS coronary angiograms with computerized image processing to obtain more equivalent measures for additional between-vessel comparisons of risk factor effects.

**Summary**

A significant per-patient therapy effect has been observed with colesteRol-niacin-plus-diabetes therapy in the femoral arteries. Total variance of CEA change in CLAS was as predicted from pilot studies, but measurement variation was larger. A per-segment analysis demonstrated a significant therapy effect in segments with moderately severe atherosclerosis and in proximal femoral segments.

**References**


**KEY WORDS** • atherosclerosis • computer-estimated atherosclerosis • clinical trials
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